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* * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 15:24:20 ON 19 NOV 2003

=> fil .bec,fsta
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
      ESBIOBASE, BIOTECHNO, WPIDS, FSTA' ENTERED AT 15:24:36 ON 19 NOV 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

12 FILES IN THE FILE LIST

=> s fructosyltransferase# or fructosyl transferase# or inulinsucrase# or
levansucrase# or (inulin or levan)(w)sucrase#
FILE 'MEDLINE'
           140 FRUCTOSYLTRANSFERASE#
           200 FRUCTOSYL
         45341 TRANSFERASE#
            17 FRUCTOSYL TRANSFERASE#
                 (FRUCTOSYL(W)TRANSFERASE#)
             0 INULINSUCRASE#
           229 LEVANSUCRASE#
          7557 INULIN
           386 LEVAN
          3008 SUCRASE#
            16 (INULIN OR LEVAN)(W)SUCRASE#
L1          377 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'SCISEARCH'
           275 FRUCTOSYLTRANSFERASE#
           292 FRUCTOSYL
         37901 TRANSFERASE#
            91 FRUCTOSYL TRANSFERASE#
                 (FRUCTOSYL(W)TRANSFERASE#)
             1 INULINSUCRASE#
           295 LEVANSUCRASE#
          2904 INULIN
           413 LEVAN
          1795 SUCRASE#
             9 (INULIN OR LEVAN)(W)SUCRASE#
L2          600 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'LIFESCI'
           104 FRUCTOSYLTRANSFERASE#
            93 "FRUCTOSYL"
         12565 TRANSFERASE#
            24 FRUCTOSYL TRANSFERASE#
                 ("FRUCTOSYL"(W)TRANSFERASE#)
             0 INULINSUCRASE#
           164 LEVANSUCRASE#
           675 INULIN
           244 LEVAN
           367 SUCRASE#
             6 (INULIN OR LEVAN)(W)SUCRASE#
L3          272 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'BIOTECHDS'
           115 FRUCTOSYLTRANSFERASE#
```

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            119 FRUCTOSYL
           2699 TRANSFERASE#
             51 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
              0 INULINSUCRASE#
            182 LEVANSUCRASE#
            409 INULIN
            201 LEVAN
             83 SUCRASE#
             10 (INULIN OR LEVAN)(W)SUCRASE#
L4          322 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'BIOSIS'
            300 FRUCTOSYLTRANSFERASE#
            442 FRUCTOSYL
          70175 TRANSFERASE#
            148 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
              0 INULINSUCRASE#
            284 LEVANSUCRASE#
           6647 INULIN
            723 LEVAN
           3366 SUCRASE#
             92 (INULIN OR LEVAN)(W)SUCRASE#
L5          707 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'EMBASE'
            120 FRUCTOSYLTRANSFERASE#
            115 "FRUCTOSYL"
          34159 TRANSFERASE#
              9 FRUCTOSYL TRANSFERASE#
                  ("FRUCTOSYL"(W)TRANSFERASE#)
              1 INULINSUCRASE#
            216 LEVANSUCRASE#
           7167 INULIN
            394 LEVAN
           1886 SUCRASE#
              7 (INULIN OR LEVAN)(W)SUCRASE#
L6          322 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'HCAPLUS'
            503 FRUCTOSYLTRANSFERASE#
            647 FRUCTOSYL
          44892 TRANSFERASE#
            117 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
              2 INULINSUCRASE#
            502 LEVANSUCRASE#
           8785 INULIN
           1085 LEVAN
           3439 SUCRASE#
             78 (INULIN OR LEVAN)(W)SUCRASE#
L7         1023 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'NTIS'
              2 FRUCTOSYLTRANSFERASE#
              2 FRUCTOSYL
           1128 TRANSFERASE#
              0 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
```

```
              0 INULINSUCRASE#
              3 LEVANSUCRASE#
             72 INULIN
             16 LEVAN
             23 SUCRASE#
              0 (INULIN OR LEVAN)(W)SUCRASE#
L8            4 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'ESBIOBASE'
            105 FRUCTOSYLTRANSFERASE#
            118 FRUCTOSYL
          28049 TRANSFERASE#
             35 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
              1 INULINSUCRASE#
            119 LEVANSUCRASE#
            954 INULIN
            135 LEVAN
            459 SUCRASE#
              4 (INULIN OR LEVAN)(W)SUCRASE#
L9          246 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'BIOTECHNO'
            122 FRUCTOSYLTRANSFERASE#
            105 FRUCTOSYL
          16291 TRANSFERASE#
             29 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
              1 INULINSUCRASE#
            201 LEVANSUCRASE#
            868 INULIN
            221 LEVAN
            485 SUCRASE#
              4 (INULIN OR LEVAN)(W)SUCRASE#
L10         316 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'WPIDS'
             28 FRUCTOSYLTRANSFERASE#
            157 FRUCTOSYL
           4328 TRANSFERASE#
             72 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
              0 INULINSUCRASE#
             19 LEVANSUCRASE#
            640 INULIN
            142 LEVAN
            105 SUCRASE#
             26 (INULIN OR LEVAN)(W)SUCRASE#
L11         125 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
                # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

FILE 'FSTA'
             54 FRUCTOSYLTRANSFERASE#
            109 FRUCTOSYL
           1969 TRANSFERASE#
             31 FRUCTOSYL TRANSFERASE#
                  (FRUCTOSYL(W)TRANSFERASE#)
              0 INULINSUCRASE#
             96 LEVANSUCRASE#
            707 INULIN
            142 LEVAN
```

```
            91 SUCRASE#
             4 (INULIN OR LEVAN)(W)SUCRASE#
L12        170 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
               # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W)SUCRASE#

TOTAL FOR ALL FILES
L13       4484 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
               # OR LEVANSUCRASE# OR (INULIN OR LEVAN)(W) SUCRASE#

=> s l13 and (lactobacillus or lactic acid bacteri?)
FILE 'MEDLINE'
         10758 LACTOBACILLUS
         28341 LACTIC
       1208871 ACID
        544109 BACTERI?
          1819 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L14          6 L1 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'SCISEARCH'
          9823 LACTOBACILLUS
         20834 LACTIC
        948693 ACID
        295462 BACTERI?
          5757 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L15         10 L2 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'LIFESCI'
          5528 LACTOBACILLUS
          6513 "LACTIC"
        266817 "ACID"
        165869 BACTERI?
          2537 LACTIC ACID BACTERI?
                 ("LACTIC"(W)"ACID"(W)BACTERI?)
L16          6 L3 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'BIOTECHDS'
          2450 LACTOBACILLUS
          5069 LACTIC
        106428 ACID
        104808 BACTERI?
          2632 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L17         10 L4 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'BIOSIS'
         15548 LACTOBACILLUS
         27296 LACTIC
       1128015 ACID
       1227904 BACTERI?
          5159 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
LI8          8 L5 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'EMBASE'
          8125 LACTOBACILLUS
         33052 "LACTIC"
       1179304 "ACID"
        401416 BACTERI?
          1919 LACTIC ACID BACTERI?
                 ("LACTIC"(W)"ACID"(W)BACTERI?)
L19          9 L6 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)
```

```
FILE 'HCAPLUS'
         19137 LACTOBACILLUS
         83328 LACTIC
       3746123 ACID
        512868 BACTERI?
          8268 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L20           13 L7 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'NTIS'
           110 LACTOBACILLUS
           561 LACTIC
         43109 ACID
         18111 BACTERI?
            33 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L21             0 L8 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'ESBIOBASE'
          3440 LACTOBACILLUS
          4848 LACTIC
        266860 ACID
        148627 BACTERI?
          1852 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L22             4 L9 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'BIOTECHNO'
          4945 LACTOBACILLUS
          8234 LACTIC
        343953 ACID
        187946 BACTERI?
          2086 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L23             7 L10 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'WPIDS'
          3293 LACTOBACILLUS
         13609 LACTIC
        811003 ACID
         89661 BACTERI?
          2015 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L24             6 L11 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'FSTA'
          9013 LACTOBACILLUS
         14608 LACTIC
        110342 ACID
         59965 BACTERI?
          6410 LACTIC ACID BACTERI?
                 (LACTIC(W)ACID(W)BACTERI?)
L25             6 L12 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

TOTAL FOR ALL FILES
L26            85 L13 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

=> s (fructan or levan or inulin)(5a)(mak###### or produc? or synthes?)
FILE 'MEDLINE'
           176 FRUCTAN
           386 LEVAN
          7557 INULIN
        252801 MAK######
       1115920 PRODUC?
```

```
        438552 SYNTHES?
L27        225 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'SCISEARCH'
           603 FRUCTAN
           413 LEVAN
          2904 INULIN
        280214 MAK######
       1513801 PRODUC?
        763143 SYNTHES?
L28        447 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'LIFESCI'
           126 FRUCTAN
           244 LEVAN
           675 INULIN
         46391 MAK######
        458495 PRODUC?
        129451 SYNTHES?
L29        231 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'BIOTECHDS'
            88 FRUCTAN
           201 LEVAN
           409 INULIN
          9338 MAK######
        187765 PRODUC?
         27295 SYNTHES?
L30        286 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'BIOSIS'
           790 FRUCTAN
           723 LEVAN
          6647 INULIN
        168514 MAK######
       1521685 PRODUC?
        596391 SYNTHES?
L31        639 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'EMBASE'
           332 FRUCTAN
           394 LEVAN
          7167 INULIN
        220965 MAK######
       1077909 PRODUC?
        536735 SYNTHES?
L32        258 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
               ?)

FILE 'HCAPLUS'
          1080 FRUCTAN
          1085 LEVAN
          8785 INULIN
        522318 MAK######
       3760233 PRODUC?
        782396 PRODN
       4144115 PRODUC?
                 (PRODUC? OR PRODN)
       1330592 SYNTHES?
```

```
L33        1210 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

FILE 'NTIS'
              3 FRUCTAN
             16 LEVAN
             72 INULIN
         115966 MAK######
         358118 PRODUC?
          41314 SYNTHES?
L34           5 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

FILE 'ESBIOBASE'
            269 FRUCTAN
            135 LEVAN
            954 INULIN
          54128 MAK######
         449202 PRODUC?
         155712 SYNTHES?
L35         205 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

FILE 'BIOTECHNO'
            225 FRUCTAN
            221 LEVAN
            868 INULIN
          34182 MAK######
         389713 PRODUC?
         168437 SYNTHES?
L36         210 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

FILE 'WPIDS'
            131 FRUCTAN
            142 LEVAN
            640 INULIN
         577591 MAK######
        2009920 PRODUC?
         110207 SYNTHES?
L37         163 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

FILE 'FSTA'
            140 FRUCTAN
            142 LEVAN
            707 INULIN
          16880 MAK######
         269297 PRODUC?
          10787 SYNTHES?
L38         298 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

TOTAL FOR ALL FILES
L39        4177 (FRUCTAN OR LEVAN OR INULIN)(5A)(MAK###### OR PRODUC? OR SYNTHES
                ?)

=> s l13(5a)l39
FILE 'MEDLINE'
L40          28 L1 (5A)L27

FILE 'SCISEARCH'
L41          53 L2 (5A)L28
```

```
FILE 'LIFESCI'
L42          23 L3 (5A)L29

FILE 'BIOTECHDS'
L43          29 L4 (5A)L30

FILE 'BIOSIS'
L44          69 L5 (5A)L31

FILE 'EMBASE'
L45          24 L6 (5A)L32

FILE 'HCAPLUS'
L46         110 L7 (5A)L33

FILE 'NTIS'
L47           0 L8 (5A)L34

FILE 'ESBIOBASE'
L48          34 L9 (5A)L35

FILE 'BIOTECHNO'
L49          31 L10(5A)L36

FILE 'WPIDS'
L50          14 L11(5A)L37

FILE 'FSTA'
L51          26 L12(5A)L38

TOTAL FOR ALL FILES
L52         441 L13(5A) L39

=> s (126 or l52) not 2002-2003/py
FILE 'MEDLINE'
        991032 2002-2003/PY
L53          25 (L14 OR L40) NOT 2002-2003/PY

FILE 'SCISEARCH'
       1827168 2002-2003/PY
L54          43 (L15 OR L41) NOT 2002-2003/PY

FILE 'LIFESCI'
        160237 2002-2003/PY
L55          22 (L16 OR L42) NOT 2002-2003/PY

FILE 'BIOTECHDS'
         40027 2002-2003/PY
L56          31 (L17 OR L43) NOT 2002-2003/PY

FILE 'BIOSIS'
        913924 2002-2003/PY
L57          67 (L18 OR L44) NOT 2002-2003/PY

FILE 'EMBASE'
        817898 2002-2003/PY
L58          24 (L19 OR L45) NOT 2002-2003/PY

FILE 'HCAPLUS'
       1933475 2002-2003/PY
L59          87 (L20 OR L46) NOT 2002-2003/PY

FILE 'NTIS'
         20286 2002-2003/PY
```

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L60          0 (L21 OR L47) NOT 2002-2003/PY

FILE 'ESBIOBASE'
        520737 2002-2003/PY
L61          26 (L22 OR L48) NOT 2002-2003/PY

FILE 'BIOTECHNO'
        227210 2002-2003/PY
L62          28 (L23 OR L49) NOT 2002-2003/PY

FILE 'WPIDS'
       1916368 2002-2003/PY
L63          14 (L24 OR L50) NOT 2002-2003/PY

FILE 'FSTA'
         38588 2002-2003/PY
L64          21 (L25 OR L51) NOT 2002-2003/PY

TOTAL FOR ALL FILES
L65         388 (L26 OR L52) NOT 2002-2003/PY

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    19.49      19.70

STN INTERNATIONAL LOGOFF AT 15:30:27 ON 19 NOV 2003
```

	L #	Hits	S arch Text	DBs	Time Stamp
1	L1	1283	fructosyltransferase$1 or fructosyl adj transferase$1 or $sucrase	USPAT; US-PGPUB	2003/11/19 14:50
2	L2	21	1 same (lactobacillus or lactic adj acid adj bacteri$8)	USPAT; US-PGPUB	2003/11/19 14:50
3	L3	142	1 same (fructan or levan or inulin)	USPAT; US-PGPUB	2003/11/19 14:51
4	L4	77	1 same ((fructan or levan or inulin) near5 (mak$6 or produc$8 or synthes$8))	USPAT; US-PGPUB	2003/11/19 14:52
5	L5	93	2 or 4	USPAT; US-PGPUB	2003/11/19 14:53

US-PAT-NO: 6583275

DOCUMENT-IDENTIFIER: US 6583275 B1

TITLE: Nucleic acid sequences and expression system relating to Enterococcus faecium for diagnostics and therapeutics

DATE-ISSUED: June 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Doucette-Stamm; Lynn A.	Framingham	MA	N/A	N/A
Bush; David	Somerville	MA	N/A	N/A

APPL-NO: 09/ 107532

DATE FILED: June 30, 1998

PARENT-CASE:

This application claims priority of U.S. provisional applications No. 60/051,571, filed Jul. 2, 1997; and No. 60/085,598 filed May 14, 1998, all of which are hereby incorporated herein by reference in their entirety.

US-CL-CURRENT: 536/23.1, 435/243 , 435/320.1 , 435/325 , 435/6 , 536/24.3 , 536/24.32

ABSTRACT:

The invention provides isolated polypeptide and nucleic acid sequences derived Enterococcus faecium that are useful in diagnosis and therapy of pathological conditions; antibodies against the polypeptides; and methods for the production of the polypeptides. The invention also provides methods for the detection, prevention and treatment of pathological conditions resulting from bacterial infection.

34 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (41):

24400077_c2_44 1394 5048 1473 490 809 1.10E-80 [ac:p40714] [gn:esca] [or:escherichia coli] [ec:3.2.1.26] [de:sucrose-6-phosphate hydrolase, (**sucrase**) (invertase)] [sp:p40714] [db:swissprot] 24400317_c1_21 1395 5049 1035 344 73 0.076 [ac:q58263] [gn:mtrg:mj0853] [or:methanococcus jannaschii] [ec:2.1.1.86] [de:methyl transferase 13 kd subunit)] [sp:q58263]

[db:swissprot] 24401035_c1_48 1396 5050 2343 780 154 3.00E-15 [ln:pip501aa] [ac:139769] [or:plasmid pip501] [sr:plasmid pip501 (strain pva1702) dna] [db:genpept-bct] [de:plasmid pip501 (from streptococcus) genes, six complete codingregions.] [nt:orf5] [le:4794] [re:6755] [di:direct] 24406387_c2_57 1397 5051 915 304 1303 4.90E-133 [ln:efentijo] [ac:y16413] [pn:transposase] [gn:orf4] [or:enterococcus faecium] [db:genpept-bct] [de:enterococcus faecium enti and entj genes and two open reading frames.] [nt:author-given protein sequence is in conflict with] [le:1311] [re:2072] [di:direct 24406502_c1_41 1398 5052 321 106 377 6.50E-35 [ln:af029727] [ac:af029727] [or:enterococcus faecium] [db:genpept-bct] [de:enterococcus faecium insertion sequence is1485, complete sequence.] [nt:putative; orfa] [le:76] [re:366] [di:direct] 24406667_c3_25 1399 5053 1062 353 996 1.70E-100 [ac:h69636] [pn:nad(p)h-dependent glycerol-3-phosphate dehydrogenase gpsa] [gn:gpsa] [or:bacillus subtilis] [db:pir] 24406687_c3_20 1400 5054 1326 441 1420 2.00E-145 [ac:p34038] [gn:pyk] [or:**lactobacillus** delbrueckii] [sr:,subspbulgaricus] [ec:2.7.1.40] [de:pyruvate kinase,] [sp:p34038] [db:swissprot] 24406952_c1_9 1401 5055 603 200 167 1.20E-12 [ac:p76219:p77229] [gn:ydjx] [or:escherichia coli] [de:hypothetical 27.9 kd protein in xtha- gdha intergenic region] [sp:p76219:p77229] [db:swissprot] 24407318_c1_11 1402 5056 885 294 890 2.80E-89 [ac:g69879] [pn:1-serine dehydratase homolog ylpa] [gn:ylpa] [or:bacillus subtilis] [db:pir] 24407762_c3_157 1403 5057 321 106 399 3.00E-37 [ln:af029727] [ac:af029727] [or:enterococcus faecium] [db:genpept-bct] [de:enterococcus faecium insertion sequence is1485, complete sequence.] [nt:putative; orfa] [le:76] [re:366] [di:direct] 24407762_c3_25 1404 5058 321 106 399 3.00E-37 [ln:af029727] [ac:af029727] [or:enterococcus faecium] [db:genpept-bct] [de:enterococcus faecium insertion sequence is1485, complete sequence.] [nt:putative; orfa] [le:76] [re:366] [di:direct] 24407762_f2_16 1405 5059 321 106 405 7.10E-38 [ln:af029727] [ac:af029727] [or:enterococcus faecium] [db:genpept-bct] [de:enterococcus faecium insertion sequence is1485, complete sequence.] [nt:putative; orfa] [le:76] [re:366] [di:direct] 24407785_f1_4 1406 5060 384 127 417 3.80E-39 [ac:p46899:p70969] [gn:rplr] [or:bacillus subtilis] [de:50s ribosomal protein 118] [sp:p46899:p70969] [db:swissprot] 24407813_c1_77 1407 5061 1155 384 1230 2.70E-125 [ac:p39148] [gn:glya:glyc:ipc-34d] [or:bacillus subtilis] [ec:2.1.2.1] [de:(shmt)] [sp:p39148] [db:swissprot] 24407827_f3_5 1408 5062 465 154 234 6.30E-19 [ac:g69992] [pn:spore cortex protein homolog ytgp] [gn:ytgp] [or:bacillus subtilis] [db:pir] 24407832_f1_9 1409 5063 876 291 694 1.70E-68 [ac:c70040] [pn:plant-metabolite dehydrogenase homolog yvgn] [gn:yvgn] [or:bacillus subtilis] [db:pir] 24407962_c3_133 1410 5064 1101 366 1269 2.00E-129 [ac:p76043:p78306] [gn:ycjq] [or:escherichia coli] [de:intergenic region] [sp:p76043:p78306] [db:swissprot] 24408187_c3_34 1411 5065 285 94 60 0.23 [ac:p39506] [gn:y14c:frd.1] [or:bacteriophage t4] [de:hypothetical 9.5 kd protein in frd-gp32 intergenic region] [sp:p39506] [db:swissprot] 24408212_f1_7 1412 5066 348 115 262 1.00E-22 [ac:q45399] [gn:cela] [or:bacillus stearothermophilus] [ec:2.7.1.69] [de:(ec 2.7.1.69)] [sp:q45399] [db:swissprot] 24408438_c3_130 1413 5067 2133 710 263 3.40E-19 [ln:u93872] [ac:u93872] [or:kaposi's sarcoma-associated herpesvirus] [sr:kaposi's sarcoma- associated herpesvirus - human herpesvirus 8] [db:genpept-vr1] [de:kaposi's sarcoma-associated herpesvirus glycoprotein m, dnareplication protein, glycoprotein, dna 24408500_f1_4 1414 5068 552 183 78 0.15 [ln:af025396] [ac:af025396] [gn:orf15x3] [or:vibrio anguillarum] [db:genpept-bct] [de:vibrio anguillarum rfb region, partial sequence.] [nt:orf15x3; function unknown] [le:11267] [re:11653] [di:direct]

24409642_c2_121 1415 5069 294 97 364 1.60E-33 [ac:p19775] [gn:tnp] [or:staphylococcus aureus] [de:transposase for insertion sequence element is256 in transposon tn4001] [sp:p19775] [db:swissprot] 24410902_c2_19 1416 5070 315 105 60 0.23 [ac:p46190] [gn:rpso] [or:mycoplasma hyorhinis] [de:30s ribosomal protein s15 (fragment)] [sp:p46190] [db:swissprot] 24410902_c2_62 1417 5071 816 271 114 0.00019 [ac:q02150] [or:lactococcus lactis] [sr:,subsplactis:streptococcus lactis] [de:hypothetical 31.3 kd protein in hisie 3'region (orf13)] [sp:q02150] [db:swissprot] 24410902_f2_72 1418 5072 762 253 108 0.00082 [ac:q02150] [or:lactococcus lactis] [sr:,subsplactis:streptococcus lactis] [de:hypothetical 31.3 kd protein in hisie 3'region (orf13)] [sp:q02150] [db:swissprot] 24410925_c1_203 1419 5073 324 107 66 0.057 [ac:f47758] [pn:reverse transcriptase (copia-like retrotransposon)] [or:liriodendron chinense] [db:pir] 24412582_c1_170 1420 5074 528 175 613 6.40E-60 [ln:efplsep1g] [ac:x96976] [pn:transposase] [gn:tnp1062] [or:enterococcus faecalis] [db:genpept-bct] [de:e.faecalis plasmid dna sep1 gene, 4068bp.] [le:2496] [re:3455] [di:complement] 24412902_c2_41 1421 5075 315 104 66 0.057 [ac:c69333] [pn:hypothetical protein af0667] [or:archaeoglobus fulgidus] [db:pir] 24413400_c3_60 1422 5076 1431 476 178 5.60E-13 [ac:p06153:p15239] [or:bacteriophage phi-105] [de:immunity repressor protein] [sp:p06153:p15239] [db:swissprot] 24414086_c2_35 1423 5077 765 254 712 2.10E-70 [ac:g69762] [pn:two-component response regulator [yelk] homolog yclj] [gn:yclj] [or:bacillus subtilis] [db:pir] 24414160_c3_151 1424 5078 1179 392 79 0.021 [ac:s66396] [pn:integrin beta 1 chain isoform d] [cl:integrin beta chain] [or:homo sapiens] [sr:, man] [db:pir] 24414202_f3_26 1425 5079 1560 519 571 1.80E-55 [ac:s74833] [pn:hypothetical protein s110855] [or:synechocystis sp.] [sr:pcc 6803, , pcc 6803] [sr:pcc 6803,] [db:pir] 24414207_c2_57 1426 5080 1005 334 630 1.00E-61 [ac:jc5310] [pn:galactose repressor] [gn:galr] [or:streptococcus mutans] [db:pir] 24414717_c1_52 1427 5081 612 203 814 3.20E-81 [ac:p12047] [gn:purb:pure] [or:bacillus subtilis] [ec:4.3.2.2] [de:adenylosuccinate lyase, (adenylosuccinase) (asl)] [sp:p12047] [db:swissprot] 24414717_f3_124 1428 5082 1497 498 608 2.20E-59 [ac:p39301] [gn:sgat] [or:escherichia coli] [de:sgat protein] [sp:p39301] [db:swissprot]

US-PAT-NO: 6617156

DOCUMENT-IDENTIFIER: US 6617156 B1

TITLE: Nucleic acid and amino acid sequences relating to Enterococcus faecalis for diagnostics and therapeutics

DATE-ISSUED: September 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Doucette-Stamm; Lynn A.	Framingham	MA	01701	N/A
Bush; David	Somerville	MA	02144	N/A

APPL-NO: 09/ 134000

DATE FILED: August 13, 1998

PARENT-CASE:

This application claims priority of U.S. Provisional application No. 60/055,778, filed Aug. 15, 1997, all of which is hereby incorporated herein by reference in its entirety.

US-CL-CURRENT: 435/320.1, 435/252.3 , 435/6 , 435/69.1 , 536/23.7 , 536/24.32

ABSTRACT:

The invention provides isolated polypeptide and nucleic acid sequences derived from Enterococcus faecalis that are useful in diagnosis and therapy of pathological conditions; antibodies against the polypeptides; and methods for the production of the polypeptides. The invention also provides methods for the detection, prevention and treatment of pathological conditions resulting from bacterial infection.

19 Claims, 0 Drawing figures

Exemplary Claim Number: 1,5,14

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (18):

393 76 0.98 [OR:Penicillium chrysogenum] [PN:orotidine-5'-phosphate decarboxylase,] [GN:pyrG] contig439 203200_c3_12 834 4239 270 90 323 2.90E-29 [AC:JH0204] [OR:Enterococcus faecalis] [PN:hypothetical 30.5K protein] contig439 25429640_f2_2 835 4240 744 247 605 7.00E-106 [AC:JC5007] [OR:Lactococcus lactis] [PN:transposase (insertion sequence IS1297)]

contig439 31646887_c1_8 836 4241 219 72 164 2.00E-12 [AC:JC1262]
[OR:Lactococcus lactis subsp. lactis] [PN:hypothetical 4.5K protein]
contig439 24648527_c2_10 837 4242 201 66 72 0.067 [AC:X97263] [OR:Lactococcus
lactis] [GN:aBIR] contig439 5086088_c1_7 838 4243 987 328 1126 2.30E-114
[SP:Q06115] [OR:**LACTOBACILLUS** PLANTARUM] [GN:CBH] [DE:HYDROLASE) (CBAH)
(BILE
SALT HYDROLASE)] contig439 3947263_f2_4 839 4244 426 142 668 8.00E-66
[AC:U17153] [OR:Enterococcus faecalis] [PN:transposase] contig439
13808206_f3_6 840 4245 246 81 164 2.00E-12 [AC:JC5008] [OR:Lactococcus lactis]
[PN:hypothetical 6.5K protein (insertion sequence IS1297)] contig44
26211592_c3_4 841 4246 642 213 875 9.30E-88 [SP:P36399] [OR:STREPTOCOCCUS
SALIVARIUS] [GN:UPP] [DE:PYROPHOSPHORYLASE) (UPRTASE)] contig44
35797061_f1_2
842 4247 240 79 contig440 1171936_c3_15 843 4248 315 104 74 0.007
[AC:L31763] [OR:Dichelobacter nodosus] [PN:virulence-associated protein I]
[GN:vapI] [NT:putative] contig440 197151_f3_5 844 4249 312 103 118 1.50E-07
[AC:U46134] [OR:Bacillus subtilis] [PN:putative transcriptional regulator]
[GN:slr] [NT:Slr; positive regulator of competence development] contig440
6147312_f3_6 845 4250 480 159 63 0.9999 [SP:Q09947] [OR:CAENORHABDITIS
ELEGANS] [GN:F12A10.6] [DE:HYPOTHETICAL 14.4 KD PROTEIN F12A10.6 IN
CHROMOSOME II] contig440 799092_f3_7 846 4251 597 198 78 0.068 [SP:P52117]
[OR:VIBRIO CHOLERAE] [GN:SMPA] [DE:SMALL PROTEIN A HOMOLOG] contig440
36135252_f1_1 847 4252 303 100 61 0.97 [SP:P39615] [OR:BACILLUS SUBTILIS]
[GN:UNG] [DE:URACIL- DNA GLYCOSYLASE,] contig440 16834442_f3_8 848 4253 1233
410 242 1.10E-18 [OR:Lactococcus lactis] [PN:integrase] [GN:int] contig440
24883402_c3_11 849 4254 477 158 572 1.20E-55 [SP:P13375] [OR:BACILLUS
STEAROTHERMOPHILUS] [GN:PGIA] [DE:ISOMERASE A)] contig440 32678443_c1_9 850
4255 333 110 185 7.10E-14 [SP:P13376] [OR:BACILLUS STEAROTHERMOPHILUS]
[GN:PGIB] [DE:ISOMERASE B)] contig441 25633317_f1_1 851 4256 2913 970 292
9.30E-25 [OR:Acidaminococcus fermentans] [PN:hgdC protein] contig442
13679637_f2_4 852 4257 2889 962 625 2.90E-61 [AC:L20670] [OR:Streptococcus
pneumoniae] [NT:alternative truncated translation product from] contig443
250777_c1_3 853 4258 183 60 50 0.91 [AC:M32362] [OR:Clostridium
cellulolyticum] [NT:protein of unknown function] contig443 24492330_f3_1 854
4259 1737 578 824 6.40E-118 [AC:X65164] [OR:Streptococcus gordonii]
[PN:fibronectin-binding protein-like protein A] [GN:flpA] contig444
16304530_f2_2 855 4260 486 161 546 6.80E-53 [SP:P13522] [OR:STREPTOCOCCUS
MUTANS] [GN:SCRB] [DE:SUCROSE-6-PHOSPHATE HYDROLASE, (**SUCRASE**)
(INVERTASE)]
contig444 24510762_f3_4 856 4261 1008 335 1035 1.00E-104 [AC:U46902]
[OR:Streptococcus mutans] [PN:ScrR] [GN:scrR] [NT:regulator of scrB
expression; sucrose regulator;] contig444 24275312_c1_5 857 4262 366 121 134
3.10E-09 [SP:P13976] [OR:ESCHERICHIA COLI] [GN:PEMK] [DE:PEMK PROTEIN]
contig444 23634838_c2_6 858 4263 276 91 130 8.20E-09 [SP:P18534]
[OR:ESCHERICHIA COLI] [GN:CHPR] [DE:PEMI- LIKE PROTEIN 1 (MAZE PROTEIN)]
contig445 6917753_c1_9 859 4264 885 294 108 0.00083 [AC:U29378]
[OR:Caenorhabditis elegans] [GN:F08C6.4] [NT:similar to erythrocyte band 7
integral membrane] contig445 161452_f3_4 860 4265 876 291 567 4.00E-55
[AC:U58210] [OR:Streptococcus thermophilus] [NT:orf1091] contig445
14484465_c1_8 861 4266 546 181 243 5.60E-20 [OR:Enterococcus faecalis]
[PN:probable pheromone binding proteinpheromone responsive gene Z protein]
[GN:prgZ] contig445 35742152_c3_12 862 4267 216 71 114 4.80E-06 [AC:D28859]
[OR:Enterococcus faecalis] [PN:TraC] [GN:traC] contig445 16125682_c2_11 863

4268 189 62 96 0.00041 [OR:Enterococcus faecalis] [PN:pheromone cAD1 binding protein precursor] [GN:traC] contig445 34569416_c2_10 864 4269 252 83 218 2.60E-17 [OR:Streptococcus "equisimilis"] [PN:hyaluronate synthase precursor] [GN:has] contig445 24407713_c1_7 865 4270 657 218 210 2.10E-16 [SP:P26906] [OR:BACILLUS SUBTILIS] [GN:DPPE] [DE:DIPEPTIDE-BINDING PROTEIN DPPE PRECURSOR] contig446 24641550_f2_4 866 4271 801 266 68 0.99995 [AC:U16732] [OR:Subterranean clover stunt virus] [GN:SCSV3] contig446 26601642_c2_20 867 4272 213 70 99 0.0001 [OR:Homo sapiens] [PN:transmembrane copper transporting P-type ATPase] contig446 24725711_c2_19 868 4273 753 250 530 1.10E-50 [AC:U42410] [OR:Proteus mirabilis] [PN:heavy-metal transporting P- type ATPase] contig446 4820391_c1_15 869 4274 276 91 79 0.044 [AC:U42410] [OR:Proteus mirabilis] [PN:heavy-metal transporting P- type ATPase] contig446 29473133_c3_24 870 4275 1095 364 678 7.00E-67 [SP:P32113] [OR:ENTEROCOCCUS FAECALIS] [GN:COPA] [DE:POTASSIUM/COPPER-TRANSPORTING ATPASE A,] contig446 24664812_c2_17 871 4276 690 229 247 4.90E-20 [AC:U42410] [OR:Proteus mirabilis] [PN:heavy-metal transporting P- type ATPase] contig446 414126_c2_16 872 4277 483 160 297 1.60E-26 [OR:Enterococcus hirae] [PN:regulatory protein copY] [GN:copY] contig446 3297325_c1_14 873 4278 861 286 640 7.40E-63 [SP:P26235] [OR:ENTEROCOCCUS HIRAE] [GN:NAPA] [DE:NA(+)/H(+) ANTIPORTER] contig447 24808441_c3_23 874 4279 1236 411 401 1.60E-37 [SP:P55340] [OR:BACILLUS SUBTILIS] [GN:ECSB] [DE:PROTEIN ECSB] contig447 2047187_c2_20 875 4280 951 316 816 1.70E-81 [SP:P55339] [OR:BACILLUS SUBTILIS] [GN:ECSA] [DE:ABC- TYPE TRANSPORTER ATP-BINDING PROTEIN ECSA] contig447 26773442_f2_8 876 4281 444 147 412 1.10E-38 [AC:Y14077] [OR:Bacillus subtilis] [PN:Hypothetical protein] [GN:yhaE] [NT:Similarity to the Hit family of proteins] contig447 976577_f2_9 877 4282 213 70 61 0.62 [AC:U08008] [OR:Metapenaeus ensis] [PN:tropomyosin] [NT:a stop codon immediately follows the last] contig447 11128437_f3_13 878 4283 213 70 68 0.045 [SP:P27183] [OR:SYNECHOCYSTIS SP] [GN:ATPG] [DE:ATP SYNTHASE B' CHAIN, (SUBUNIT II)] contig447 391288_f1_6 879 4284 1107 368 376 7.00E-35 [SP:Q02473] [OR:**LACTOBACILLUS** PARACASEI] [GN:PRTM] [DE:PROTEASE MATURATION PROTEIN PRECURSOR] contig447 6769677_c2_17 880 4285 219 72 54 0.9999 [SP:P24212] [OR:ESCHERICHIA COLI] [GN:SBMA] [DE:SBMA PROTEIN] contig447 5907938_c1_14 881 4286 231 76 237 3.80E-20 [AC:Y14078] [OR:Bacillus subtilis] [PN:Hypothetical protein] [GN:yhaM] [NT:similarity to CMP-binding-factor-1 (cbf1) from] contig448 26173161_f3_2 882 4287 276 91 76 0.13 [SP:Q12263] [OR:SACCHAROMYCES CEREVISIAE] [GN:GIN4] [DE:SERINE/THREONINE-PROTEIN KINASE GIN4,] contig448 707_f1_1 883 4288 2730 910 114 0.0054 [OR:Bacillus sphaericus]

PGPUB-DOCUMENT-NUMBER: 20030175911

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175911 A1

TITLE: Process for the preparation of L-amino acids with amplification of the zwf gene

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hans, Stephen	Osnabruek		DE	
Bathe, Brigitte	Salzkotten		DE	
Reth, Alexander	Bielefeld		DE	
Thierbach, Georg	Bielefeld		DE	
Kreutzer, Caroline	Melle		DE	
Mockel, Bettina	Dusseldorf		DE	

APPL-NO: 10/ 336049

DATE FILED: January 3, 2003

RELATED-US-APPL-DATA:

child 10336049 A1 20030103

parent continuation-in-part-of 10091342 20020306 US PENDING

child 10091342 20020306 US

parent continuation-in-part-of 09531269 20000320 US ABANDONED

US-CL-CURRENT: 435/115, 435/252.3

ABSTRACT:

The invention relates to a process for the preparation of L-amino acids by the fermentation of coryneform bacteria. The process involves: fermenting an L-amino acid-producing bacteria in which at least the zwf gene is amplified; concentrating the L-amino acid in the medium or in the cells of the bacteria; and isolating the L-amino acid produced.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a continuation-in-part of U.S. Ser. No. 10/091,342, filed on Mar. 6, 2002, which is a continuation-in-part of U.S. Ser. No. 09/531,269, filed Mar. 20, 2000.

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Detail Description Paragraph - DETX (222):

[0219] The plasmid pK18mobsacB_zwf(A243T), like the starting plasmid pK18mobsacB, contains, in addition to the kanamycin resistance gene, a copy of the sacB gene which codes for levan **sucrase** from Bacillus subtilis. The expression which can be induced by sucrose leads to the formation of **levan sucrase, which catalyses the synthesis of the product levan,** which is toxic to C. glutamicum. Only those clones in which the integrated plasmid pK18mobsacB_zwf(A243T) has excised as the consequence of a second recombination event therefore grow on LB agar containing sucrose. Depending on the position of the second recombination event with respect to the mutation site either allele exchange (i.e., incorporation of the mutation) occurs or the original copy (i.e. the wild type gene) remains in the chromosome of the host.

PGPUB-DOCUMENT-NUMBER: 20030186940

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030186940 A1

TITLE: Method for preparing a polydispersed saccharide composition and resulting polydispersed saccharide composition

PUBLICATION-DATE: October 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
De Leenheer, Leen	Tervuren		BE	
Booten, Karl	Geetbeets		BE	

APPL-NO: 10/ 317545

DATE FILED: December 12, 2002

RELATED-US-APPL-DATA:

child 10317545 A1 20021212

parent division-of 09230769 19990201 US PENDING

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
BE	09600676	1996BE-09600676	August 1, 1996
	PCT/BE97/00087		July 25, 1997

US-CL-CURRENT: 514/61, 536/123

ABSTRACT:

A method for preparing a polydispersed saccharide composition in which a fructan-containing material is dissolved in water prior to partial enzymatic treatment of the fructans.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (24):

[0024] The DP of an **inulin produced** by microorganisms may vary up to values of the order of 60,000. Such an **inulin is, for example, synthesized** from saccharose by Aspergillus sydowi conidia in the presence of L-cysteine, as described in the article "Characteristics and Applications of a Polyfructan Synthesized from Sucrose by Aspergillus sydowi conidia" (T. Harada et al., Food Hydrocolloids, Vol. 7, No. 1, pp. 23-28 (1993)). The **production of a**

"bacterial" inulin by a fructosyltransferase from Streptococcus mutans is described in "Genetic and Antigenic Comparison of Streptococcus mutans **Fructosyltransferase** and Glucan-binding Protein" (J. Aduse-Opoku, FEMS Microbiology Letters 59, pp. 279-282 (1989)).

Summary of Invention Paragraph - BSTX (28):

[0028] In the case where the fructans are inulin, an enzymatic preparation having an endo-inulinase activity is used. Such preparations are known and can be obtained i.a. from cultures of Penicillium, Aspergillus, Fusarium or Chrysosporium (see also the document "The **production of Fructooligosaccharides from Inulin** or Sucrose Using Inulinase or **Fructosyltransferase** from Aspergillus ficuum" (Denpun Kagaku, Vol. 36, No. 2, pp. 103-111 (1989)), incorporated herein by reference).

PGPUB-DOCUMENT-NUMBER: 20030190711

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030190711 A1

TITLE: Novel insulin synthase and process for producing inulin by using the same

PUBLICATION-DATE: October 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wada, Tadashi	Shizuoka		JP	
Ohguchi, Masao	Shizuoka		JP	

APPL-NO: 10/ 311318

DATE FILED: December 30, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	195245/2000	2000JP-195245/2000	June 28, 2000

PCT-DATA:
APPL-NO: PCT/JP01/01133
DATE-FILED: Feb 16, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/101, 435/200 , 435/252.31 , 435/320.1 , 435/69.1 , 536/123 , 536/23.2

ABSTRACT:

The present invention relates to a novel inulin synthase having a function and a substrate specificity of acting on sucrose to produce inulin, but not acting on kestose, maltose, lactose, trehalose and cellobiose; and a process for producing inulin comprising the step of allowing the synthase, a culture fluid or cultured cells of a microorganism producing the synthase, or a treated product thereof to contact with sucrose to produce inulin.

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Summary of Invention Paragraph - BSTX (8):

[0006] On the other hand, the above-mentioned higher plants from which inulin can be extracted obviously contain an enzyme for **producing inulin**, and it has already been shown by M. Luscher et al. (FEBS Letter 385, 39 (1996)) that **inulin is produced** from sucrose using an enzyme that is extracted from such a plant. This mechanism is driven by the cooperative action of two types of enzymes: sucrose 1-**fructosyltransferase** (SST), a sucrose which performs transfer of fructosyl between sucroses, and .beta.-(2.fwdarw.1) fructan 1-**fructosyltransferase** (FFT), a .beta.-(2.fwdarw.1) fructan which transfers fructose moieties between fructans having a degree of polymerization of 3 or more.

Summary of Invention Paragraph - BSTX (15):

[0013] Hidaka et al. have proposed a method for **producing linear fructan** having .beta.-(2.fwdarw.1) linkages by allowing **fructosyltransferase** produced by microorganisms belonging to the genus Aspergillus or Fusarium to act on sucrose (JP Patent Publication (Unexamined Application) No. 55-40193). However, the **fructan produced** in this case is an oligosaccharide wherein 1 to 4 molecules of fructose are bound to sucrose, so that it is defined as a substance different from inulin in molecular size.

US-PAT-NO: 6635460

DOCUMENT-IDENTIFIER: US 6635460 B1

TITLE: Fructosyltransferases

DATE-ISSUED: October 21, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 604958

DATE FILED: June 28, 2000

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	00201872	May 25, 2000

US-CL-CURRENT: 435/193, 536/23.2

ABSTRACT:

The present invention describes two novel proteins having **fructosyltransferase** activity. Both enzymes are derived from lactobacilli, which are food-grade microorganisms with the Generally Recognized As Safe (GRAS) status. One of these proteins **produces an inulin and fructo-oligosaccharides, while the other produces a levan**. According to the invention lactobacilli capable of **producing an inulin** and/or a levan and/or fructo-oligosaccharides using one or both of the **fructosyltransferases** can be used as a probiotic or a symbiotic.

5 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

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Abstract Text - ABTX (1):

The present invention describes two novel proteins having

fructosyltransferase activity. Both enzymes are derived from lactobacilli, which are food-grade microorganisms with the Generally Recognized As Safe (GRAS) status. One of these proteins **produces an inulin and fructo-oligosaccharides, while the other produces a levan**. According to the invention lactobacilli capable of **producing an inulin** and/or a levan and/or fructo-oligosaccharides using one or both of the **fructosyltransferases** can be used as a probiotic or a symbiotic.

Brief Summary Text - BSTX (6):

The exopolysaccharides produced by LAB can be divided in two groups, heteropolysacchrides and homopolysaccharides; these are synthesized by totally different mechanisms. The former consist of repeating units in which residues of different types of sugars are present and the latter consist of one type of monosaccharide. The synthesis of heteropolysaccharides by **lactic acid bacteria,** including lactobacilli, has been studied extensively in recent years. Considerably less information is available on the synthesis of homopolysaccharides from lactobacilli, although some studies have been performed. Homopolysacoharides with fructose as the constituent sugar can be divided into two groups, inulins and levans. Inulins consist of 2,1-lined .beta.-fructofuranoside residues, whereas levans consist of 2,6-linked .beta.-fructofuranoside residues. Both can be linear or branched, The size of bacterial levans can vary from 20 kDa up to several MDa. There is limited information on the synthesis of levans, In most detail this synthesis has been studied in Zymomonas mobilis and in Bacillus species, Within **lactic acid bacteria, fructosyltransferases** have only been studied in streptococci. So far no **fructosyltransferases** have been reported in lactobacilli.

Brief Summary Text - BSTX (9):

Two novel genes encoding enzymes having **fructosyltransferase** activity have now been found in **Lactobacillus** reuteri, and their amino acid sequences have been determined. These are the first two enzymes identified in a **Lactobacillus** species capable of **producing a fructan, One of the eves is an inulosucrase which produces** a high molecular weight (>10.sup.7 Da) fructan containing .beta.(2-1) linked fructosyl units and fructo-oligosaccharides, while the other is a **levansucrase which produces a fructan** containing .beta.(2-6) linked fructosyl units. The invention thus pertains to the enzymes, to DNA encoding them, to recombinant cells containing such DNA and to their use in producing carbohydrates, as defined in the appending claims.

Brief Summary Text - BSTX (11):

It was found according to the invention that one of the novel **fructosyltransferases** (FTFA; an **inulosucrase) produces a high molecular weight inulin** with .beta.(2-1) linked fructosyl units and fructo-oligosaccharides. The fructo-oligosaccharides synthesis was also observed in certain **Lactobacillus** strains, in particular in certain strains of **Lactobacillus** reuteri. However, the inulin has not been found in **Lactobacillus** reuteri culture supernatants, but only in extracts of E. coli cells expressing the above-mentioned **fructosyltransferase**. This **inulosucrase** consists of either 798 amino acids (2394 nucleotides) or 789 amino acids (2367 nucleotides) depending

on the potential start codon used. The molecular weight (MW) deduced of the amino acid sequence of the latter form is 86 kDa and its isoelectric point is 4.51, at pH 7.

Brief Summary Text - BSTX (14):

Fructosyltransferases have been found in several bacteria such as Zymomonas mobilis, Erwinia amylovora, Acetobacter amylovora, Bacillus polymyxa, Bacillus amyloliquefaciens, Bacillus stearothermophilus, and Bacillus subtilis. In **lactic acid bacteria** this type of enzyme previously has only been found in some streptococci. Most bacterial **fructosyltransferases** have a molecular mass of 50-100 kDa (with the exception of the **fructosyltransferase** found in Streptococcus salivarius which has a molecular mass of 140 kDa). Amino acid sequence alignment revealed that the novel **inulosucrase** of lactobacilli has high homology with **fructosyltransferases** originating from Gram positive bacteria, in particular with Streptococcus enzymes. The highest homology (FIG. 2) was found with the SacB enzyme of Streptococcus mutans Ingbritt A (62% identity within 539 amino acids).

Brief Summary Text - BSTX (17):

A recombinant host cell, such as a mammalian (with the exception of human), plant, animal, fungal or bacterial cell, containing one or more copies of the nucleic acid construct mentioned above is an additional subject of the invention. The **inulosucrase** gene (staring at nucleotide 41) has been cloned in an E. coli expression vector under the control of an ara promoter in E. coli Top10. E. coli Top10 cells expressing the recombinant **inulosucrase** hydrolysed sucrose and **synthesized fructan** material. SDS-PAGE of arabinose induced E. coli Top10 cell extracts suggested that the recombinant **inulosucrase** has a molecular weight of 80-100 kDa, which is in the range of other known **fructosyltransferases** and in line with the molecular weight of 86 kDa deduced of the amino acid sequence depicted in FIG. 1.

Brief Summary Text - BSTX (18):

The invention further covers an **inulosucrase** according to the invention which, in the presence of sucrose, **produces a inulin** having .beta.(2-1)-linked D-fructosyl units and fructo-oligosaccharides. Two different types of fructans, inulins and levans, exist in nature. Surprisingly, the novel **inulosucrase** expressed in E. coli Top10 cell synthesizes a high molecular weight (>10.sup.7 Da) inulin and fructo-oligosaccharides, while in **Lactobacillus** reuteri culture supernatants, in addition to the fructo-oligosaccharides, a levan and not an inulin is found, This discrepancy can have several explanations: the **inulosucrase** gene may be silent in **Lactobacillus** reuteri, or may not be expressed in **Lactobacillus** reuteri under the conditions tested, or the **inulosucrase** may only **synthesize fructo-oligosaccharides in its natural host, or the inulin** polymer may be degraded shortly after synthesis, or may not be secreted and remains cell-associated, or the **inulosucrase** may have different activities in **Lactobacillus** reuteri and E. coli Top10 cells.

Brief Summary Text - BSTX (19):

It was furthermore found according to the invention that certain lactobacilli, in particular **Lactobacillus** reuteri, possess another **fructosyltransferase, a levansucrase** (FTFB), in addition to the **inulosucrase** described above. The N-terminal amino acid sequence of the **fructosyltransferase** purified from **Lactobacillus** reuteri supernatant was found to be (portion of SEQ ID NO: 6) QVESNNYNGVAEVNTERQANGQI. Furthermore, three internal sequences were identified, namely (SEQ ID NOS 7, 8 & 9, respectively in order of appearance) (M)(A)HLDVWDSWPVQDP(V), NAGSIFGT(K), V(E)(E)VYSPKVSTLMASDEVE. The N-terminal amino acid sequence could not be identified in the deduced **inulosucrase** sequence. Also the amino acid sequences of the three internal peptide fragments of the purified **fructosyltransferase** were not present in the putative **inulosucrase** sequence. Evidently, the **inulosucrase** gene does not encode the purified **fructosyltransferase synthesizing the levan**. The **fructan produced by the levansucrase** was identified in the **Lactobacillus** reuteri culture supernatant as a linear (2.fwdarw.6)-.beta.-D-fructofuranan with a molecular weight of 150 kDa. The purified enzyme also **produces this fructan**.

Brief Summary Text - BSTX (20):

Additionally, the invention thus covers a protein having **levansucrase** activity with an amino acid identity of at least 65%, preferably at least 75%, and more preferably at least 85%, compared to the amino acid sequence of SEQ ID NO. 2 (see FIG. 3). The second novel **fructosyltransferase produces a high molecular weight fructan** with .beta.(2-6) linked fructosyl units with sucrose or raffinose as substrate. Furthermore, the invention covers a protein or a part thereof having **levansucrase** activity containing one or more of the three internal peptide fragments and/or the N-terminal amino acid sequence shown in SEQ ID No. 2 or a part thereof hang at least 7 contiguous amino acids, preferably at least 10 contiguous amino acids, more preferably at least 12 contiguous amino acids or even at least 15 contiguous amino acids, which are identical to the corresponding part of the amino acid sequence of SEQ ID No. 2. A nucleotide sequence encoding any of the above-mentioned proteins, mutants, variants or parts thereof is a subject of the invention as well as a nucleic acid construct comprising the nucleotide sequence mentioned above operationally linked to an expression-regulating nucleic acid sequence. A recombinant host cell, such as a mammalian (with the exception of human), plant, animal, fungal or bacterial cell, containing one or more copies of the nucleic acid construct mentioned above is an additional subject of the invention. The invention further covers a protein according to the invention which, in the presence of sucrose, **produces a fructan** having .beta.(2-6)-linked D-fructosyl units.

Brief Summary Text - BSTX (21):

The invention also pertains to a process of **producing an inulin**-type and/or a levan-type of fructan as described above using **fructosyltransferases** according to the invention and a suitable fructose source such as sucrose or raffinose. The fructans may either be produced by (**Lactobacillus**) strains containing one or both **fructosyl transferases or by a fructosyltransferase** enzyme isolated by conventional means from the culture of **fructosyltransferases**-positive lactobacilli, especially a **Lactobacillus**

reuteri, or from a recombinant organism containing the **fructosyltransferase** gene or genes.

Brief Summary Text - BSTX (22):

Additionally, the invention concerns a process of producing fructo-oligosaccharides containing the characteristic structure of the fructans described above using a **Lactobacillus** strain containing one or both **fructosyltransferases** or an isolated **fructosyltransferase** according to the invention. There is a growing interest in oligosaccharides derived from homopolysaccharides, for instance for prebiotic purposes. Several fructo- and gluco-oligosaccharides are known to stimulate the growth of bifidobacteria in the human colon. Fructo-oligosaccharides produced by the **fructosyltransferase** described above are also part of the invention. Another way of producing fructo-oligosaccharides is by hydrolysis of the fructans described above. This hydrolysis can be performed by known hydrolysis methods such as enzymatic hydrolysis with enzymes such as levanase or inulinase or by acid hydrolysis. The fructo-oligosaccharides to be produced according to the invention prefarably contain at least 2, more preferably at least 3, up to about 20 anhydrofructose units, optionally in addition to one or more other (glucose, galactose, etc.) units. These fructo-oligosaccharides are useful as prebiotics, and can be administered to a mammal in need of improving the bacterial status of the colon.

Brief Summary Text - BSTX (26):

Use of a **Lactobacillus** strain capable of **producing a levan,** inulin or fructo-oligosaccharides or a mixture thereof as a probiotic, is also covered by the invention. Preferably, the **Lactobacillus** strain is also capable of producing a glucan, especially an 1,4/1,6-.alpha.-glucan as referred to above. The efficacy of some **Lactobacillus** reuteri strains as a probiotics has been demonstrated in various animals such as for instance poultry and humans. The admission of some **Lactobacillus** reuteri strains to pigs resulted in significantly lower serum total and LDL-cholesterol levels, while in children **Lactobacillus** reuteri is used as a therapeutic agent against acute diarrhea. For this and other reasons **Lactobacillus** reuteri stains, which were not reported to produce the glucans or fructans described herein, have been supplemented to commercially available probiotic products. The mode of action of **Lactobacillus** reuteri as a probiotic is still unclear. Preliminary studies indicated that gut colonization by **Lactobacillus** reuteri may be of importance. According to the invention, it was found that the mode of action of **Lactobacillus** reuteri as a probiotic may reside partly in the ability to produce polysaccharides. **Lactobacillus** strains, preferably **Lactobacillus** reuteri strains, and more preferably **Lactobacillus** reuteri strain LB 121 and other strains containing one or more **fructosyltransferase** genes encoding proteins capable of producing inulins, levans and/or fructo-oligosaccharides can thus advantageously be used as a probiotic. They can also, together with these polysaccharides, be used as a symbiotic.

Detailed Description Text - DETX (3):

Isolation of DNA from **Lactobacillus** reuteri Nucleotide Sequence Analysis of

the **Inulosucrase** (ftfA) Gene, Construction of Plasmids for Expression of the **Inulosucrase** Gene in E. coli Top10, Expression of the **Inulosucrase** Gene in E. coli Top10 and Identification of the Produced Polysaccharides Produced by the Recombinant Enzyme

Detailed Description Text - DETX (4):

General procedures for cloning, DNA manipulations and agarose gel electrophoresis were essentially as described by Sambrook et al. (1989) Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, N.Y. Restriction endonuclease digestions and ligations with T4 DNA ligase were performed as recommended by the suppliers. DNA was amplified by PCR techniques using ampliTAQ DNA polymerase (Perkin Elmer) or Pwo DNA polymerase. DNA fragments were isolated from agarose gels using the Qiagen extraction kit (Qiagen GMBH), following the instructions of the suppliers. **Lactobacillus** reuteri strain 121 (LMG 18388) was grown at 37.degree. C. in MRS medium (DIFCO) or in MRS-s medium (MRS medium containing 100 g/l sucrose instead of 20 g/l glucose). When fructo-oligosaccharides production was investigated phosphate was omitted and ammonium citrate was replaced by ammonium nitrate in the MRS-s medium. E. coli sins were grown aerobically at 37.degree. C. in LB medium, where appropriate supplemented with 50 .mu.g/ml ampicillin (for selection of recombinant plasmids) or with 0.02% (w/v) arabinose (for induction of the **inulosucrase** gene).

Detailed Description Text - DETX (6):

The **inulosucrase** gene was identified by amplification of chromosomal DNA of **Lactobacillus** reuteri with PCR using degenerated primers (5ftf, 6ftfi, and 12ftfi, see table 1) based on conserved amino acid sequences deduced from different bacterial **fructosyltransferase** genes (SacB of Bacillus amyloliquefaciens, SacB of Bacillus subtilis, Streptococcus mutans **fructosyltransferase** and Streptococcus salivarius **fructosyltransferase**, see FIG. 4) and **Lactobacillus** reuteri DNA as template. Using primers 5ftf and 6ftfi, an amplification product with the predicted size of about 234 bp was obtained (FIG. 5A). This 234 bp fragment was cloned in E. coli JM109 using the pCR2.1 vector and sequenced. Transformations were performed by electroporation using the BioRad gene pulser apparatus at 2.5 kV, 25 .mu.F and 200 .OMEGA., following the instructions of the manufacturer. Sequencing was performed according to the method of Sanger et al (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467. Analysis of the obtained sequence data confirmed that part of a **fructosyltransferase** (ftf) gene had been isolated. The 234 bp amplified fragment was used to design primers 7ftf and 8ftfi (see table 1). PCR with the primers 7ftf and 12ftfi gave a product of the predicted size of 948 bp (see FIG. 5B); its sequence showed clear similarity with previously characterized **fructosyltransferase** genes. The 948 bp amplified fragment was used to design the primers ftfAC1(i) and ftfAC2(i) (see table 1) for inverse PCR. Using inverse PCR techniques a 1438 bp fragment of the **inulosucrase** gene was generated, including the 3' end of the **inulosucrase** gene (see FIG. 5C). The remaining 5' fragment of the **inulosucrase** gene was isolated with a combination of standard and inverse PCR techniques. Briefly, **Lactobacillus** reuteri DNA was cut with restriction enzyme XhoI and ligated. PCR with the primers 7ftf and 8ftfi, using the ligation product as a template, yielded a 290 bp PCR product

which was cloned into pCR2.1 and sequenced. This revealed that primer 8ftfi had annealed a specifically as well as specifically yielding the 290 bp product (see FIG. 5D).

Detailed Description Text - DETX (7):

At this time, the N-terminal amino acid sequence of a **fructosyltransferase** enzyme (FTFB) purified from the **Lactobacillus** reuteri strain 121 was obtained. This sequence consisted of the following 23 amino acids: (portion of SEQ ID NO: 6) QVESNNYNGVAEVNTERQANGQI (see FIG. 1 and SEQ ID No. 2 in FIG. 3). The degenerated primer 19ftf (portion of SEQ ID NO: 6) (YNGVAEV) was designed on the basis of a part of this N-terminal peptide sequence and primer 20ftfi was designed on the 290 bp PCT product. PCR with primers 19ftf and 20ftfi gave a 754 bp PCT product (see FIG. 5E), which was cloned into pCR2.1 and sequenced. Both DNA strands of the entire **fructosyltransferase** gene were double sequenced. In this way the sequence of a 2.6 kb region of the **Lactobacillus** reuteri DNA, containing the **inulosucrase** gene and its surroundings were obtained.

Detailed Description Text - DETX (8):

The plasmids for expression of the **inulosucrase** gene in E. coli Top10 were constructed as described hereafter. A 2414 bp fragment, containing the **inulosucrase** gene starting at the first putative start codon at position 41, was generated by PCR, using primers ftfA1 and ftfA2i. Both primers contained suitable restriction enzyme recognition sites (a NcoI site at the 5' end of ftfA1 and a BglII site at the 3' end of ftfA2i). PCR with **Lactobacillus** reuteri DNA, Pwo DNA polymerase and primers ftfA1and ftfA2i yielded the complete **inulosucrase** gene flanked by NcoI and BglII restriction sites. The PCR product with blunt ends was ligated directly into pCRbluntII-Topo. Using the NcoI and BglII restriction sites, the putative ftfA gene was cloned into the expression vector pBAD, downstream of the inducible arabinose promoter and in frame upstream of the Myc epitope and the His tag. The pBAD vector containing the **inulosucrase** gene (pSVH101) was transformed to E. coli Top10 and used to study **inulosucrase** expression. Corrects construction of plasmid containing the complete **inulosucrase** gene was confirmed by restriction enzyme digestion analysis and by sequence analysis, showing an in frame cloning of the **inulosucrase** gene using the ribosomal binding site provided by the pBAD vector and the first putative start codon (at position 41) of **inulosucrase** (see FIG. 1).

Detailed Description Text - DETX (10):

The **fructosyltransferase** activities were determined at 37.degree. C. in reaction buffer (25 mM sodium acetate, pH 5.4, 1 mM CaCl.sub.2, 100 g/l sucrose) by monitoring the release of glucose from sucrose, by detecting fructo-oligosaccharides or by determing the amount of **fructan polymer produced** using E. coli cell free extracts or **Lactobacillus** reuteri culture supernatant as enzyme source. Sucrose, glucose and fructose were determined enzymatically using commercially available kits.

Detailed Description Text - DETX (11):

Fructan production by Lactobacillus reuteri was studied with cells grown in MRS-s medium. Product formation was also studied with cell-free extracts of E. coli containing the novel **inulosucrase** incubated in reaction buffer (1 mg protein/10 ml buffer, incubated overnight at 37.degree. C.). Fructans were collected by precipitation with ethanol. .sup.1 H-NMR spectroscopy and methylation analysis were performed as described by van Geel-Schutten et al. (1999) Appl. Environ. Microbiol. 65, 3008-3014. The molecular weights of the fructans were determined by high performance size exclusion chromatography coupled on-line with a multi angle laser light scattering and a differential refractive index detector. Fructo-oligosaccharides synthesis was studied in **Lactobacillus** reuteri culture supernatants and in extracts of E. coli cells containing the novel **inulosucrase** incubated in reaction buffer (1 mg protein/10 ml buffer, incubated overnight at 37.degree. C.). Glucose and fructose were determined enzymatically as described above and fructo-oligosaccharides produced were analyzed using a Dionex column. The incubation mixtures were centrifuged for 30 min at 10,000.times.g and diluted 1:5 in a 100% DMSO solution prior to injection on a Dionex column. A digest of inulin (DP1-20) was used as a standard. Separation of compounds was achieved with anion-exchange chromatography on a CarboPac Pa1 column (Dionex) coupled to a CarboPac PA1 guard column (Dionex). Using a Dionex GP50 pump the following gradient was generated: % eluent B is 5% (0 min); 35% (10 min); 45% (20 min); 65% (50 min); 100% (54-60 min); 5% (61-65 min). Eluent A was 0.1 M NaOH and eluent B was 0.6 M NaAc in a 0.1 M NaOH solution. Compounds were detected using a Dionex ED40 electrochemical detector with an AU working electrode and a Ag/AgCl reference-electrode with a sensitivity of 300 nC. The pulse program used was:+0.1 Volt (0-0.4 s); +0.7 Volt (0.41-0.60 s); -0.1 Volt (0.61-1.00 s). Data were integrated using a Perkin Elmer Turbochrom data integration system. A different separation of compounds was done on a cation exchange column in the calcium form (Benson BCX4). As mobile phase Ca-EDTA in water (100 ppm) was used, The elution speed was 0.4 ml/mini at a column temperature of 85.degree. C. Detection of compounds was done by a refractive index (Jasco 830-RI) at 40.degree. C. Quantification of compounds was achieved by using the software program Turbochrom (Perkin Elmer).

Detailed Description Text - DETX (21):

A **levansucrase** enzyme was purified from LB121 cultures grown on media containing maltose using ammonium sulfite precipitation and several chromatography column steps (table 2). Maltose (glucose-glucose) was chosen because both **glucansucrase and levansucrase** can not use maltose as substrate. LB121 will grow on media containing maltose but will not produce polysaccharide. From earlier experiments it was clear at even with harsh methods the **levansucrase** enzyme could not be separated from its **product levan**. These harsh methods included boiling the levan in a SDS solution and treating the levan with HCl and TFA. No levanase enzyme was commercially available for the enzymatic breakdown of levanase. Only a single **levansucrase** was detected in maltose culture supernatants. In order to prove that the enzyme purified from maltose culture supernatant is the same enzyme which is responsible for the **levan production** during growth on raffinose, biochemical and biophysical tests were performed.

Detailed Description Text - DETX (30):
FIGS. 1(1)-1(4): SEQ ID NOS 1 & 3-5; The deduced amino acid sequence of the novel **inulosucrase of Lactobacillus** reuteri (amino acid 1-789). Furthermore, the designations and orientation (<for 3' to 5' and > for 5' to 3') of the primers and the restriction enzymes used for (inverse) PCR, are shown at the right hand side. Putative start codons (ATG, at positions 41 and 68) and stop codon (TAA, at position 2435) are shown in bold. The positions of the primers used for PCR are shown in bold/underlined. The NheI restriction sites (at positions 1154 and 2592) used for inverse PCR are underlined. The primers used and their exact positions in the **inulosucrase** sequence are shown in table 1. Starting at amino acid 690, the 20 PXX repeats are underlined. At amino acid 755 the LPXTG (SEQ ID NO: 22) motif is underlined.

Detailed Description Text - DETX (32):
FIGS. 3(1) and 3(2) SEQ ID No. 2; The nucleotide sequence of a part of the novel **levansucrase of Lactobacillus** reuteri and the N-terminal (SEQ ID NO: 6) and three internal amino acid sequences of **Lactobacillus** reuteri (SEQ ID NOS 7-9).

Detailed Description Text - DETX (34):
FIG. 5: The strategy used for the isolation of the **inulosucrase** gene from **Lactobacillus** reuteri 121 chromosomal DNA.

Detailed Description Paragraph Table - DETL (2):
TABLE 2 Purification of the **Lactobacillus** reuteri LB 121 **levansucrase** (FTFB) enzyme. Protein Total Step Activity Activity Specific Purification (mg) (U) (U/mg) (fold) (%) Yield Supernatant 128 64 0.5 1 100 Ammonium sulfate 35.2 42 12 2.4 65.6 precipitation (65%) Hydroxyl apatite 1.5 30.6 20.4 40.8 47.8 Phenyl superose 0.27 23 85 170 36 Gel Filtration 0.055 10 182 360 16 MonoQ 0.0255 4 176 352 6

Claims Text - CLTX (5):
5. A process for producing an **inulosucrase,** comprising culturing a **Lactobacillus** strain containing **inulosucrase,** according to claim 1 in a culture medium, and recovering the protein from a culture medium or a cell lysate.

US-PAT-NO: 6645515

DOCUMENT-IDENTIFIER: US 6645515 B1

TITLE: Bacteriostatic composition for salmonellae

DATE-ISSUED: November 11, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 856826

DATE FILED: May 25, 2001

PARENT-CASE:

This application is a 371 application of PCT/JP99/06574 filed Nov. 25, 1999.

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JP	10-335585	November 26, 1998

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ABSTRACT:

Provided is a bacteriostatic composition for salmonellae containing, as the active ingredient, a fermented broth obtained by effecting fermentation with the use of a lactic acid bacterium belonging to the genus Leuconostoc, Streptococcus or Streptobacterium in a sucrose-containing medium, or a preparation originating in the supernatant obtained by subjecting the fermented broth to fractional precipitation from a water-miscible organic solvent.

7 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Detailed Description Text - DETX (9):

In the invention it is surmised that the desired effect can be accomplished by the following action mechanism, although not limited thereto. When the **lactic acid bacterium** is inoculated into a sucrose-containing composition and cultured, glucose among glucose and fructose as the component sugars of sucrose polymerizes into dextran, and, on the other hand, fructose is produced, but in some occasion, the fructose exists in the form of oligo- or poly-fructose as a result of polymerization by the action of **fructosyltransferase**. In addition, various substances including lactic acid and perfume substances are produced from the bacterium, and therefore, a fermented broth comprising many components probably containing lactic acid, fructose, mannitol, leucrose, cells of the **lactic acid bacterium** used for the fermentation, and other components is formed. Since it is surmised that even after this fermented broth is subjected to the fractional precipitation treatment with the organic solvent as mentioned above, the supematant fraction contains lactic acid, perfume components, fructose, mannitol, leucrose, dextran, oligo- or poly-fructose, and other culture broth components, and in some occasion, cels of the **lactic acid bacterium** used for the fermentation, the fermented broth can conveniently be used for providing the preparation of the invention. And it is surmised that by combination of these components, the preparation is harmless and enhances the taste of animals, and increases the physical condition of animals by its lowering effect of the pH of cecum dung, its inhibitory effect of invasion of the intestinal mucosa and its inhibitory effect of salmonella fixation in the salmonella attack test on chickens, and its action of inhibition of mortality and lowering of droppings pH, and exerts excellent effect in production of salmonellae-free hen's eggs, meat and cow's milk.